Data Evaluation Record for a Non-Guideline Residue Study of Thiamethoxam (CGA293343) and its Metabolite (CGA322704) with A9807C Treated Winter Oil-Seed Rape Seed in Southern, France

Citation:	Sabine Hecht-Rost, D., 2007. Thiamethoxam (CGA293343) and its Metabolite Clothianidin (CGA322704) - A Residue Study with A9807C Treated Winter Oil-Seed Rape Seed, Investigating Residues in Crop and Honeybee Products in Southern, France
PC Code:	044309, 060109, 071503,113501
CAS #:	153719-23-4
Document No.:	MRID 49158917
Guideline:	N/A
GLP Statements:	Yes
Test Formulation:	A9807C (280 g thiamethoxam L^{-1}, 8 g fludioxonil L^{-1} and 33.3 g metalxyl-M L^{-1})
Classification:	This study is classified as **SUPPLEMENTAL**. The residue data (thiamethoxam and CGA 322704 residues in winter oil-seed rape plants and honeybee products) may be used quantitatively in risk assessments. The data for the visual assessments on brood development are of limited value because the thiamethoxam formulation included two other active ingredients (systemic fungicides). In addition, the exposure time (7-8 days after exposure) was short. The study deficiencies are summarized in page 5.
Study Completion Date:	February 29, 2007
Sponsor:	Syngenta Crop Protection, LLC, Greensboro, NC
Performing Laboratory:	Eurofins-GAB GmbH, Niefern-Öschelbronn, Germany Study Number: 2032747 Report Number: 20051041/F3-BZEU
Primary Reviewer:	He Zhong, Ph.D., Biologist EPA/OPP/EFED HE ZHONG Digitally signed by HE ZHONG DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=HE ZHONG, dnQualifier=0000051219 Date: 2015.05.20 14:38:37 -04'00' Date: 03-09-2015
Secondary Reviewer:	Meghan Radtke, Ph.D., Biologist EPA/OPP/EFED MEGHAN RADTKE Digitally signed by MEGHAN RADTKE DN: c=US, o=U.S. Government, ou=USEPA, ou=Staff, cn=MEGHAN RADTKE, dnQualifier=0000040893 Date: 2015.05.21 10:28:43 -04'00' Date: 03-09-2015

Summary

The purpose of the study was to determine the magnitude of residues of thiamethoxam (CGA293343) and its metabolite CGA322704 in crop and honeybee products, following use of a flowable concentrate mixture of thiamethoxam (280g thiamethoxam L^{-1}, 8g fludioxonil L^{-1} and 33.3g metalaxyl-M L^{-1} FS formulation referred to as A9807C) as a seed treatment for winter oil-seed rape. In addition, the test and control beehives were visually inspected and the strength of the colony and presence of a healthy egg-laying queen were recorded to assess any adverse effects. The visual observation data are of limited value because the thiamethoxam formulation included two other active ingredients and the exposure time was inadequate. There was one drilling of the treated winter oil-seed rape on September 10, 2004 and the potential exposure to honeybees was evaluated in 2005. The residue data confirmed that the honeybees were exposed daily to ≤ 5ppb thiamethoxam and ≤ 6ppb metabolite CGA322704 when foraging on the winter oil-seed rape originated from the A9807C treated seed in the screened tunnels. The ranges of residues of thiamethoxam and CGA322704 for the crop and honey products are listed in **Table 1**.

Table 1. Summary of Thiamethoxam and Its Metabolite CGA322704 Residue Data

Matrix	Year	Control	Thiamethoxam (mg/Kg)	CGA322704 (mg/Kg)
Whole Plants	2005	<LOQ[1]	0.001 – 0.005	≤ 0.001
Bee Pollen	2005	<LOQ	0.001 – 0.004	< 0.001
Bee Nectar	2005	<LOQ	0.001 – 0.004	≤ 0.001
Hive Wax	2005	<LOQ	< 0.0005 (LOQ)	< 0.001 (LOQ)
Hive Nectar	2005	<LOQ	0.0006 – 0.0009	< 0.001 (LOQ)
Hive Honey	2005	<LOQ	< 0.0005 (LOQ)	< 0.001 (LOQ)
Hive Pollen	2005	<LOQ	0.001 - 0.004	0.001-0.006

[1]Limit of Quantitation (LOQ) for plants, soil, bee pollen and hive pollen is 0.0005 mg/Kg for thiamethoxam and 0.001 mg/Kg for CGA322704.

I. Study Location and Residue Sampling

Winter oil-seed rape, pre-treated with the seed treatment A9807C or untreated as a control (only treated with fungicide thiram), were sown in Midi-Pyrénéés in France on September 10, 2004 by Syngenta Agro SAS. Honeybee colonies were exposed to the flowering winter oil-seed rape in April, 2005. Honeybees were maintained in mesh-covered tunnels. The samples of whole plants and honeybee products were collected at the trial site for residue analysis. The study defines DAE0 as the first day (April 8, 2005) the hives were exposed to the thiamethoxam treated crop. The sampling schedule was summarized in **Table 2**.

Table 2. Sampling Schedule as Day after Exposure (DAE) for the Plants and Bee Products

	Year	Day after exposure (DAE) and Sample Intervals (S)[1]
Whole Plant	2005	DAE4 (S1), DAE6 (S2), DAE9 (S3)
Bee Pollen and Nectar	2005	DAE4 (S1), DAE6 (S2), DAE9 (S3)

Comb (Hive Pollen, Honey and Wax)	2005	DAE-1(S1), DAE4 (S2), DAE6 (S3), DAE9 (S4), DAE19 (S5) DAE30 (S6), DAE60 (S7), DAE90 (S8), DAE120 (S9), DAE150 (S10)

[1]The sample intervals (S1, S2, S3….) were corresponded to the DAE for each matrix and were used to track the samples from the field collection to the laboratory analysis.

II. Winter Oil-Seed Rape Seed Treatment and Application Rate

Treatment and drilling of the winter oil-seed rape crop and its maintenance until the set up of hives in the tunnels is documented in a separate report (20041365/F1-BFEU), which was not submitted to the Agency. **Table 3** is an abbreviated summary of the available application information for this study report.

Table 3. Summary of Thiamethoxam Concentration in Seed and Application Rate

	2005
Product application rate for A9807C	1.5 L / 100 kg seeds
Actual seed concentration (a.i. mg / kg seed)	4,200[1]
Actual seeding rate (kg seed / ha)	N/A[2]
Application rate (g a.i./ha)	N/A
Application rate (lb a.i./A)	N/A
Treatment (A9807C)	3 tunnels / 1 colony per tunnel
Control	1 tunnel / 1 colony per tunnel

[1] Calculated by the formula: 1.5 L/100 * 280 a.i. (g/L) * 1000

[2] For details see final report 20041365/F1-BFEU

III. Test Beehives

The trial location was near Toulouse in the region of Midi-Pyrénéés, France. Each testing hive had healthy young bees (1 queen and approximate 10,000 to 20,000 bees per colony) with two boxes (lower box = brood chamber, and upper box = honey comb box). There were at least 6 - 9 brood combs containing all brood stages and at least 9 - 10 honey and pollen combs in each colony. The bees were free of symptoms of *Nosema* and other bee disease. The colony condition was assessed prior to the introduction into the tunnels (DAE-1) and again at the end of flowering/honeybee exposure DAE9 (2005). The hives were introduced into the tunnels at the start of flowering of the winter oil-seed rape crop, in the evening of April 7, 2005 (DAE-1) before daily bee flight had started. DAE0 was the first day that the honeybees were exposed to the treated winter oil-seed rape crop. At DAE9, the colonies were relocated and maintained at the remote site to minimize the further exposure to the pesticide in Gers, Le Marteret..

IV. Brood Development Comparisons

In 2005, the test and control beehives were visually inspected and the strength of the colony and presence of a healthy egg-laying queen were recorded to assess any adverse effects after exposure to the A9807C formulation. It appears that there are no measureable effects on bee brood development between the A9807C treatment group (with the three replicate tunnels T1-

T3) and the control (tunnel C with thiram), and the pre-exposure and post exposure assessment. However, it may be premature to make a no observed adverse effect conclusion from the data because of the limited exposure and observation time (1 day pre-exposure and 9 days exposure time). The comparison of the brood development data is summarized in **Table 4**.

Table 4. Comparison of Brood Development in 2005

	T1	T2	T3	C
Pre-exposure assessment: April 7, 2005 (DAE-1)				
Strength (No. of combs covered with bees)	11	10.5	11.5	11.5
No. of combs with brood	6	8.0	9.0	9.0
Average area with eggs (%)	15	20.0	21.1	15.6
Average area with larvae (%)	11.7	10.6	7.8	10.0
Average area with pupae (capped cells)%	49.2	48.8	38.3	38.3
End of post-exposure assessment: April 17, 2005(DAE9)				
Strength (No. of combs covered with bees)	8.0	9.0	10	9.5
No. of combs with brood	7.0	7.0	7.0	8.0
Average area with eggs (%)	10.7	11.4	12.9	11.9
Average area with larvae (%)	12.9	15.7	15.7	12.5
Average area with pupae (capped cells)%	25.7	34.3	30.0	36.3

V. Sample Analysis

Bee product samples, (100 mg) were extracted by vigorous shaking with methanol:0.2% formic acid in ultra-pure water (50:50 v/v). Aliquots equivalent to 50 mg were diluted with ultra-pure water. Sample clean-up was performed by solid-phase extraction (SPE) using Oasis HLB cartridges. Plant samples (unspecified amount) were extracted with methanol:water (50:50 v/v) and then an aliquot was diluted with ultra-pure water.

Thiamethoxam and its metabolite CGA322704 residues were determined by a high performance liquid chromatography with triple quadrupole mass spectrometric detection (LC-MS/MS) by matrix matched standards in both studies. The primary ions are 211.2 (m/z) for thiamethoxam and 169.0 (m/z) for CGA322704 respectively. The limit of quantification of the method was 0.0005 mg/Kg and 0.001 mg/Kg for thiamethoxam and CGA322704, respectively, in bee nectar and hive wax, nectar and honey. The limit of quantification of the method was 0.001 mg/Kg for both thiamethoxam and CGA322704 in bee pollen, hive pollen and whole plant samples.

In general, the method satisfies the repeatability criteria with acceptable mean recoveries (70-120%) and RSDs (≤20%). The linearity is established in the calibration (y=a+bx) using external standards for thiamethoxam (e.g. hive pollen: $r^2 = 0.9987$) and CGA322704 (e.g. hive pollen: $r^2 = 0.9957$).

VI. Study Limitations

The residue study is classified as **SUPPLEMENTAL.** The residue data may be used for quantitatively in risk assessments. The data for the visual assessments on brood development are of limited value because the thiamethoxam formulation included two other active ingredients and the short exposure time. The following are the major limitations:

1) The A9807C compound mixture of three active ingredients (one systemic insecticide and two systemic fungicides) was used for the test. Thus, it is unknown if any effects (or lack of effects) were the result of thiamethoxam alone or a result of interactions among the active ingredients in the mixture.
2) The control has no replicate and has fungicide thiram for unknown reason.
3) Drilling of the winter oil-seed rape crop (= pesticide application information) and hive background data prior to the set up in the tunnels are not provided to the Agency.
4) The condition of brood development between treatment and control groups was compared only for a short exposure time (9 days).
5) The limited hive numbers (3 treatment replicates and 1 control) and huge data variation within the treatment groups limits the values of such data.
6) Data of bee mortality and abnormal foraging flight activity were not collected.
7) The residue samples were not analyzed immediately, but were stored for over 4-8 months at $\leq$ -18 °C. Since there were no matrix spike samples associated with the sample during sample collection and storage, the residue stability is uncertain. Therefore, it may be potentially underestimated the thiamethoxam and CGA322704 residue levels because of potential degradation during the sample collection and storage.
8) It is uncertain if the analytical method was validated by an independent laboratory.
9) The limit of detection (LOD) was not reported.
10) Calibration levels of standard concentrations were not provided
11) Images for mass spectrum were not included.
12) Secondary ions were not reported